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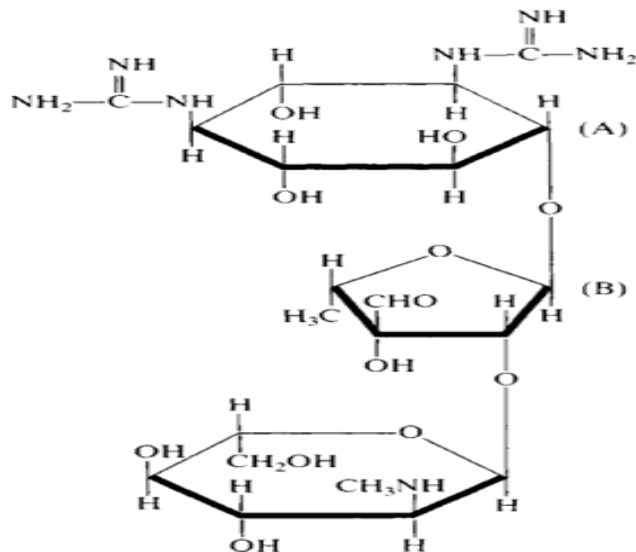
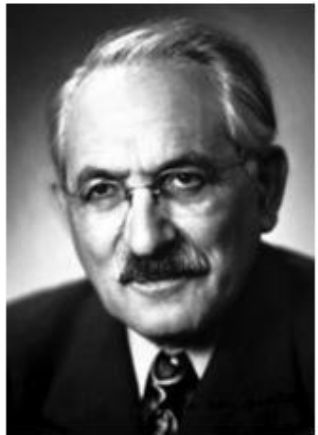
Linking animal and grassland microbiomes via manure

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Department of Biology

Soil: Antimicrobials & AMR

Waksman & Merck



Examples of AMR in environmental bacteria

Soil:

- *Streptomyces griseus* – Streptomycin APH(3'')-I, APH(6)-I
- *Stenotrophomonas maltophilia* L1/L2 carbapenemase

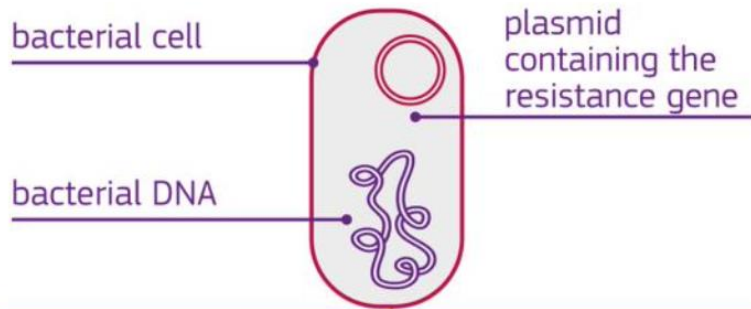
Water:

- *Shewanella* spp *bla*CTX-M

Many environmental bacteria not cultured

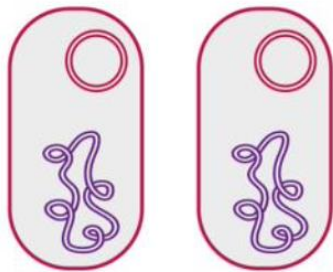
Antimicrobial Resistance movement

A: Vertical Transmission

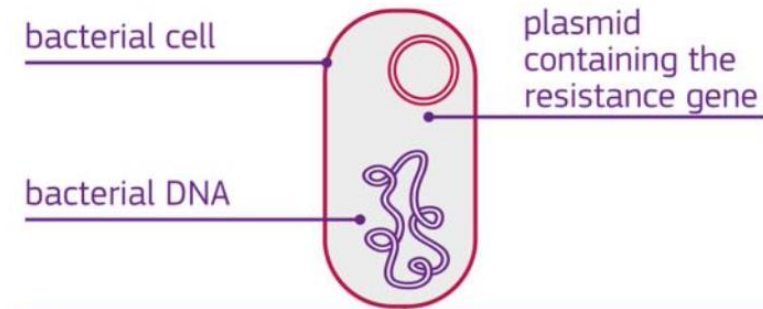


Plasmid transferred during replication to daughter cells

Daughter cells



B: Horizontal Transmission

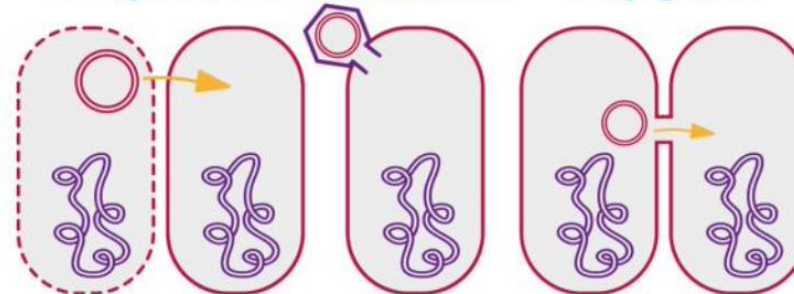


Plasmid transferring to other bacteria of the same generation

Transformation

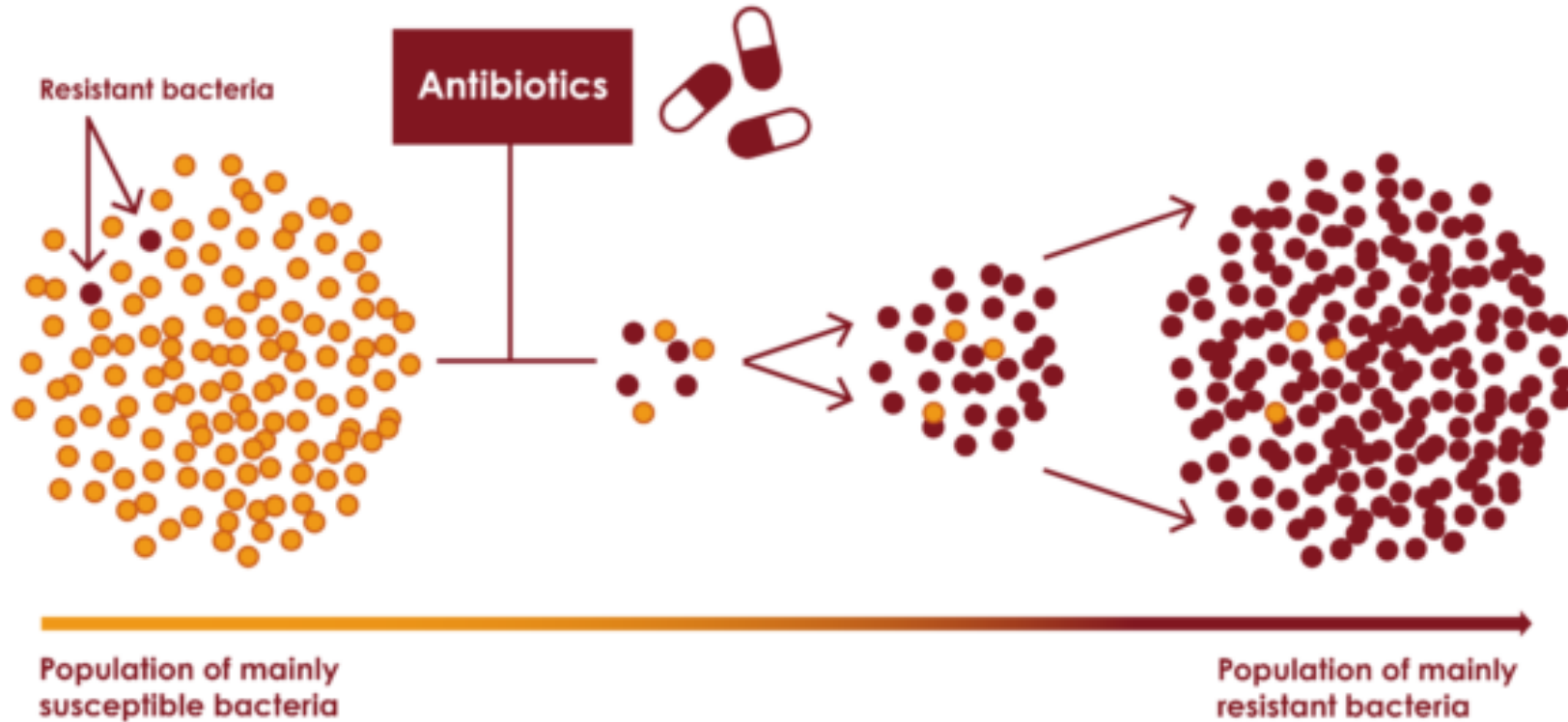
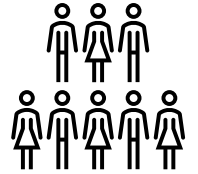
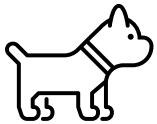
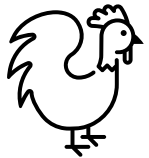
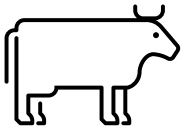
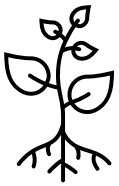
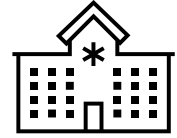
Transduction

Conjugation

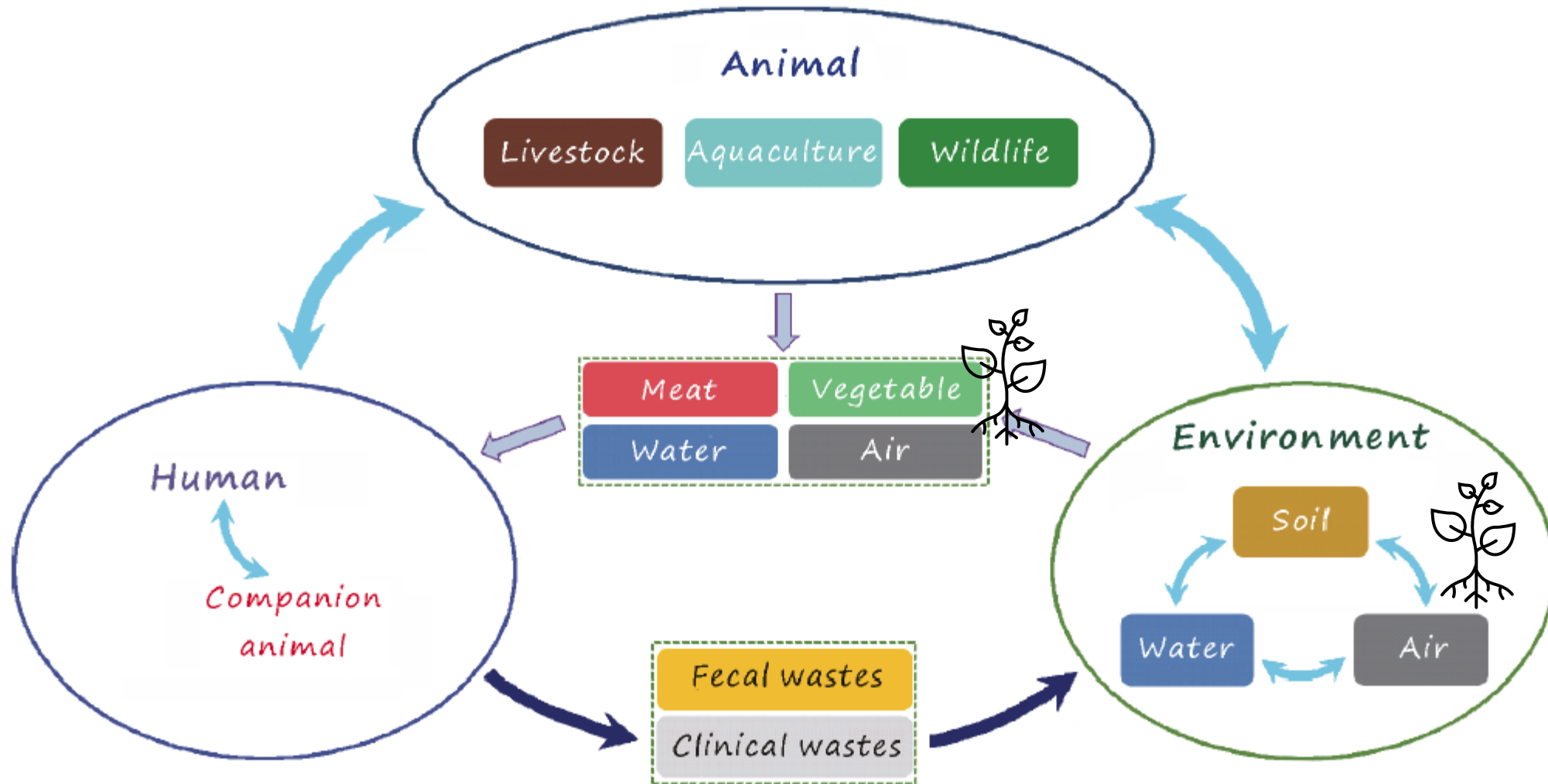


Antimicrobial Resistance Selection

Natural selection of resistant bacteria

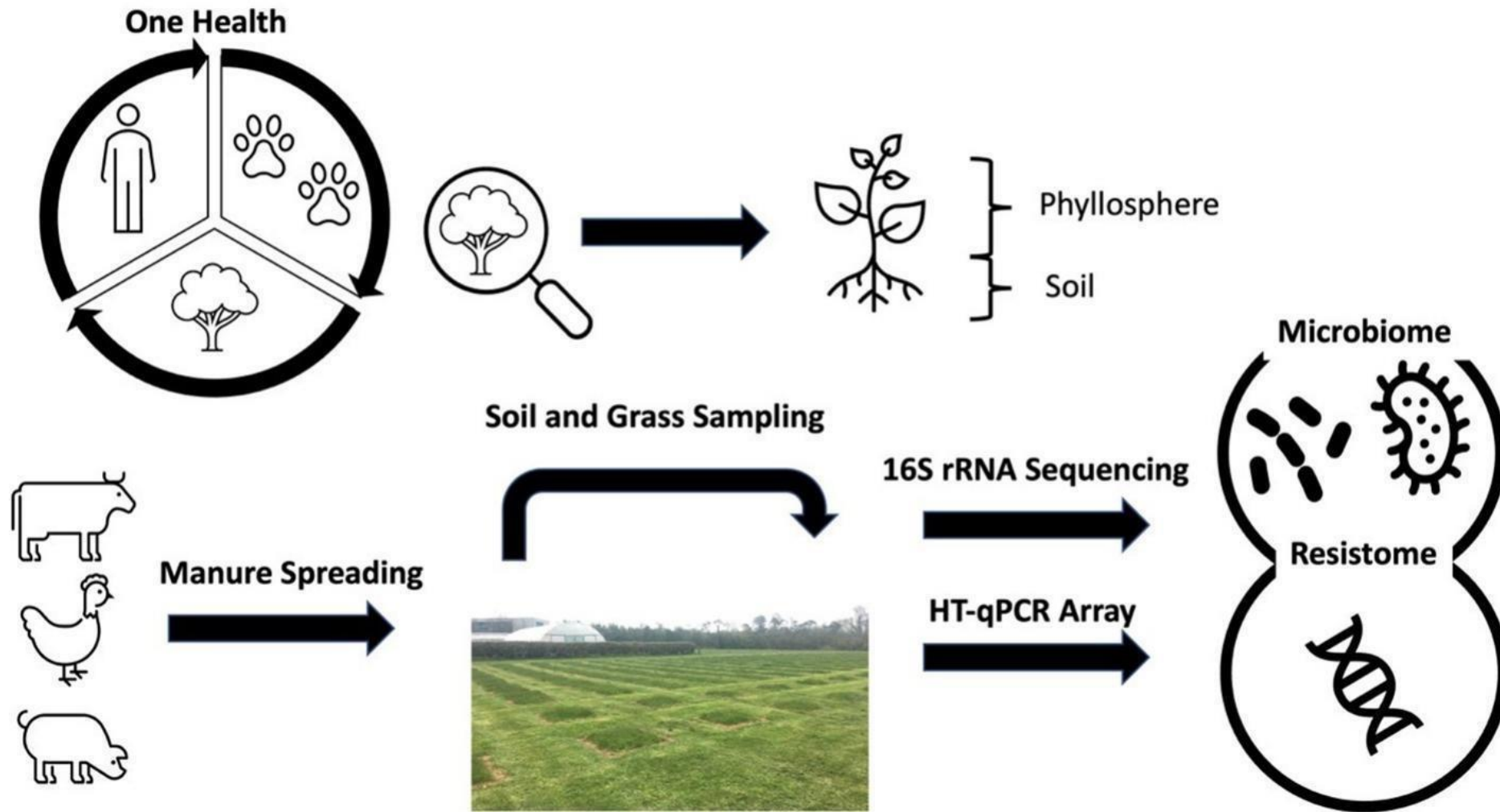


One Health



*Tiedje et al.,
Pedosphere,
2019*

Field trial experiment



Field trial overview

Samples: Manure (before application), soil and grass

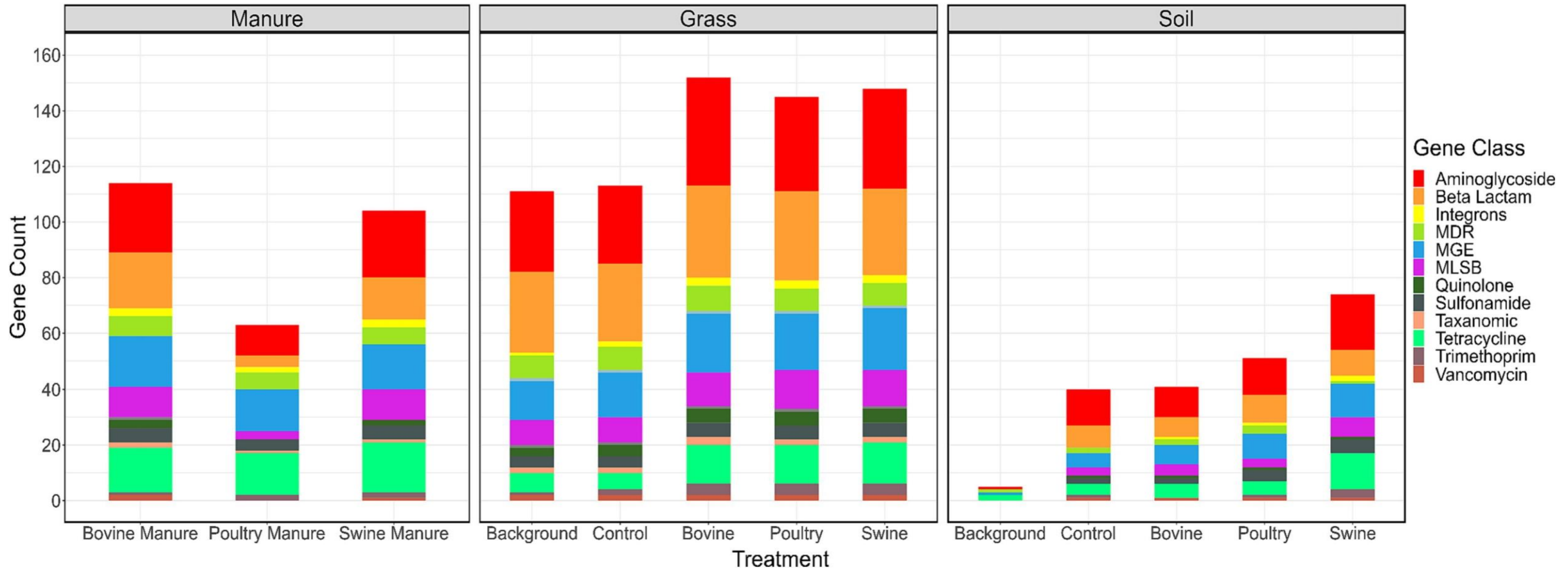


Time Point	
BM	Before Manure Added
T0	Directly after manure added
T1	1 week after manure added
T2	2 weeks after manure added
T3	3 weeks after manure added
T4	4 weeks after manure added
T5	5 weeks after manure added
T6	6 weeks after manure added
T7	10 weeks after manure added
T8	14 weeks after manure added
T9	18 weeks after manure added
	Culturing Timepoints (n=7)



AMR genes detected: Time & Source

Number of Genes Detected in Each Sample



Relative abundances AMR genes in grass

Relative abundance of

(A)ARGs

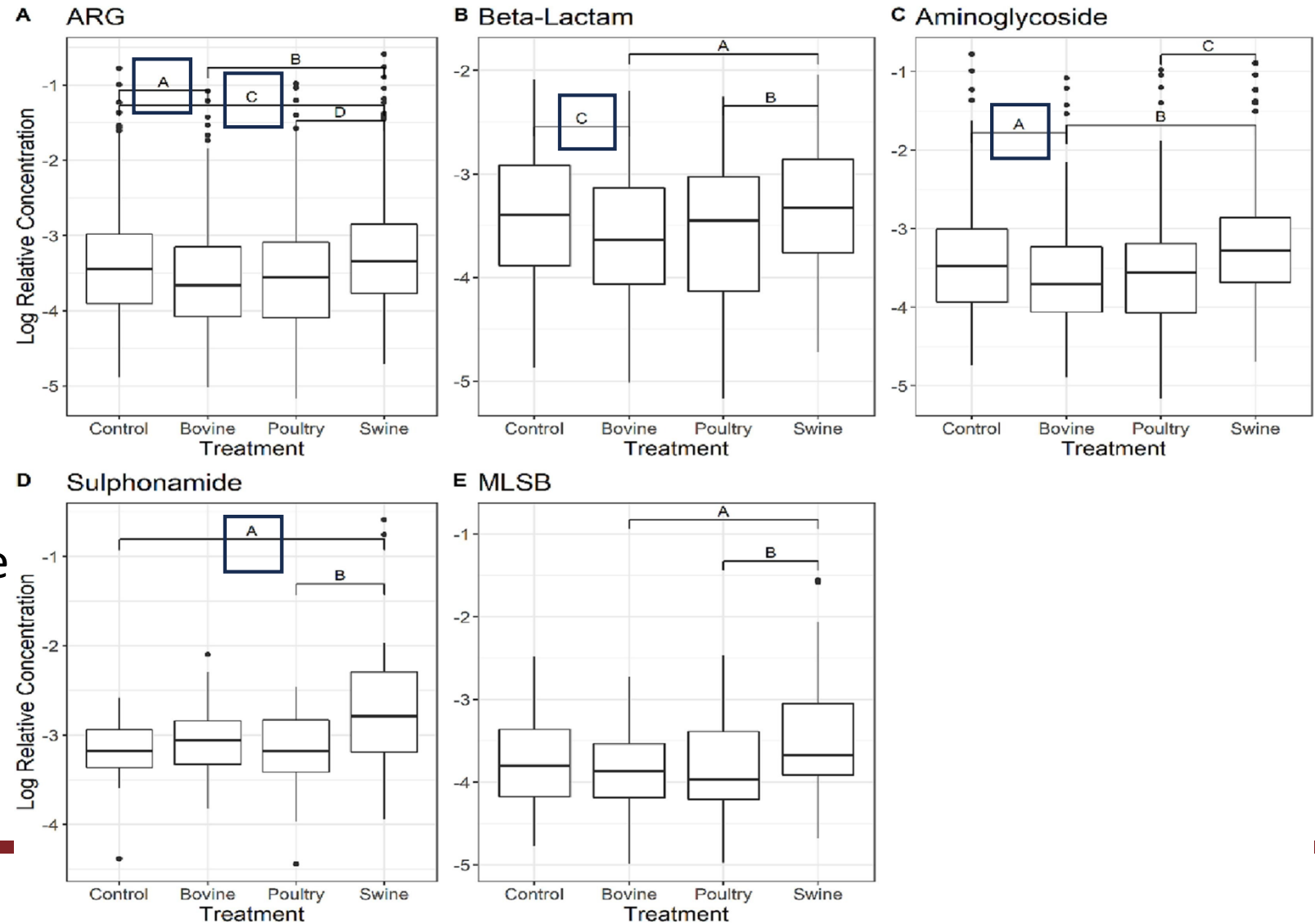
(B) β -lactam

(C)Aminoglycoside

(D)Sulphonamide

(E)MLSB

The letters A,B,C and D in the graphs indicate significance between groups ($p < 0.05$).



Relative abundances AMR genes in soil

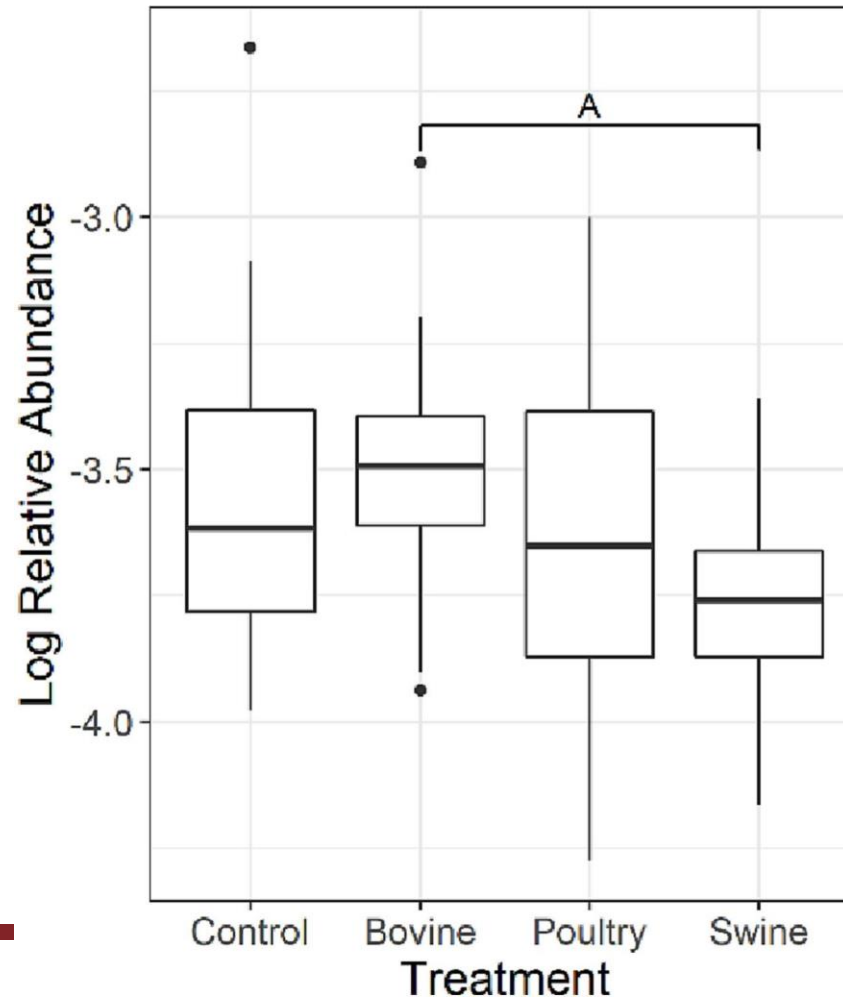
Relative abundance of

(A) Beta Lactamase

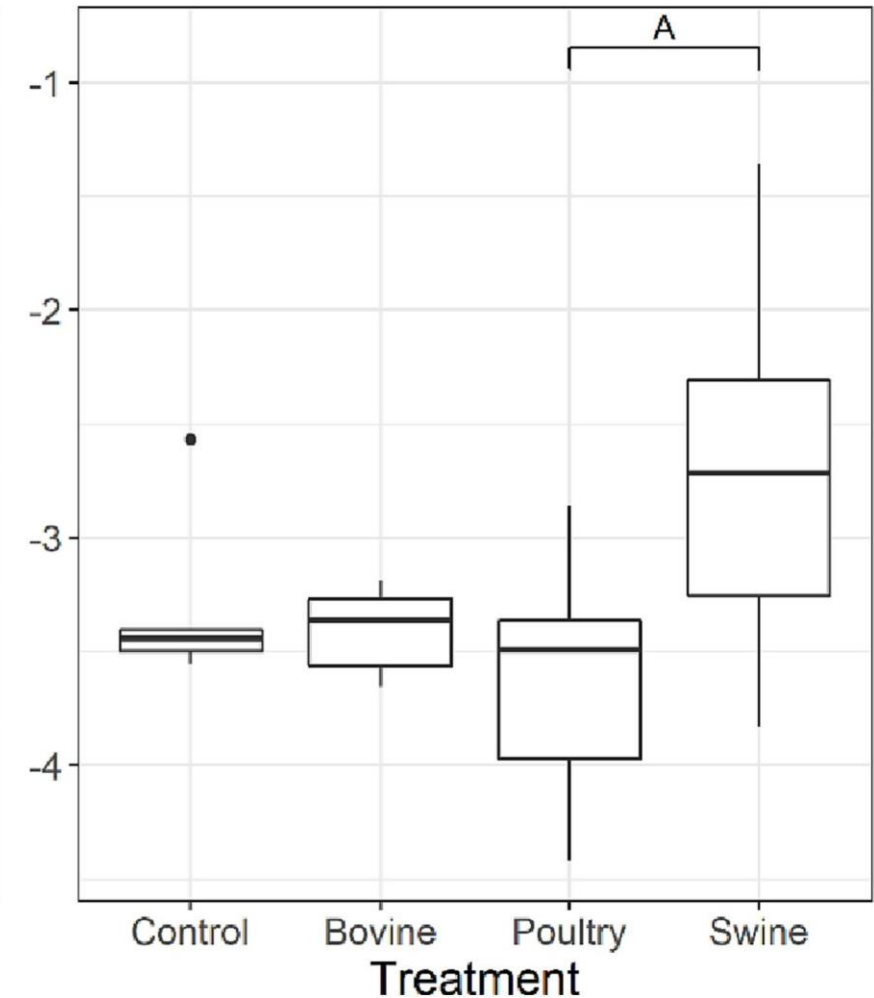
(B) Sulphonamide

The letter A indicates significance between groups ($p < 0.05$).

A Beta-Lactam



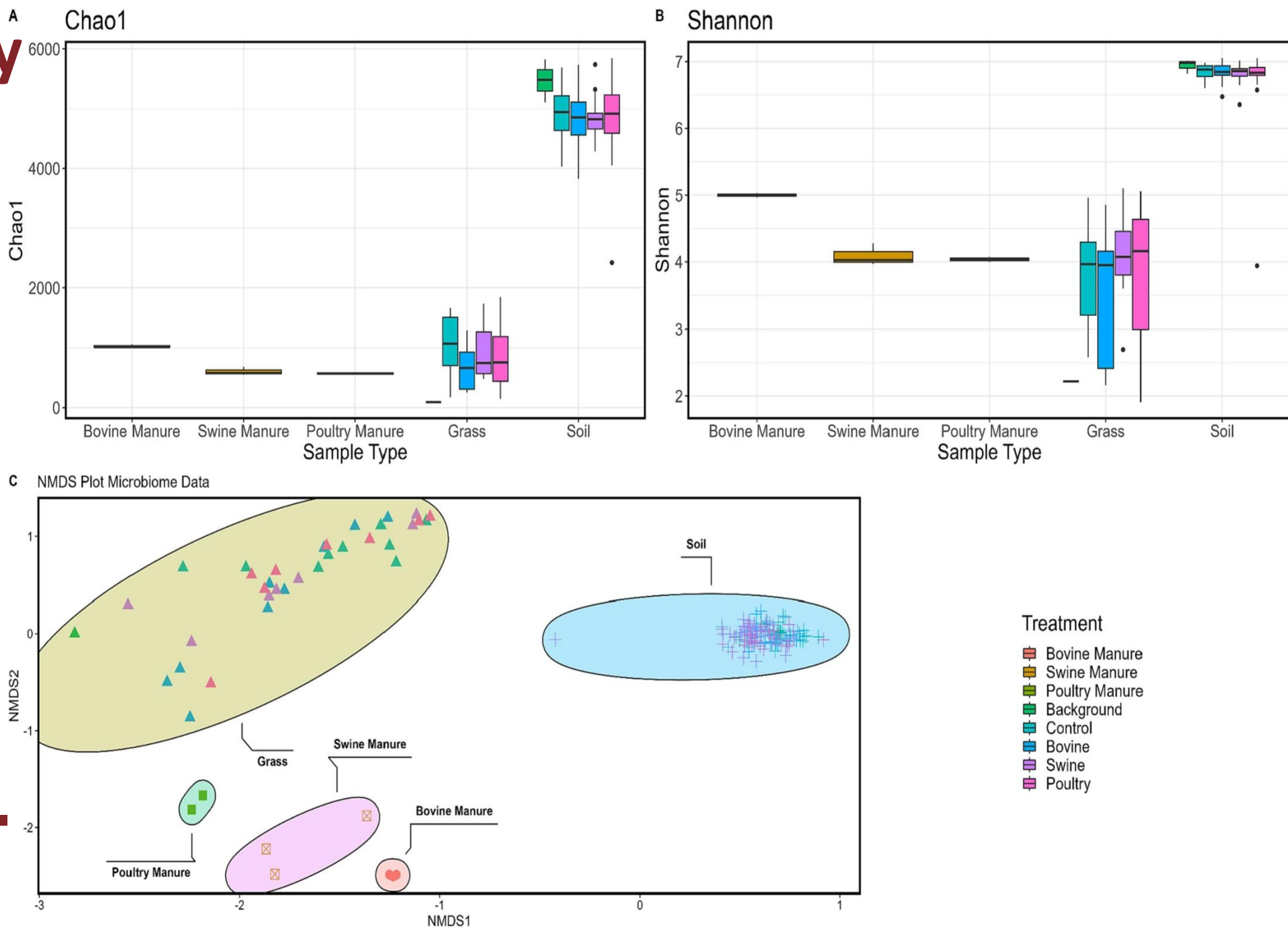
B Sulphonamide



Alpha diversity

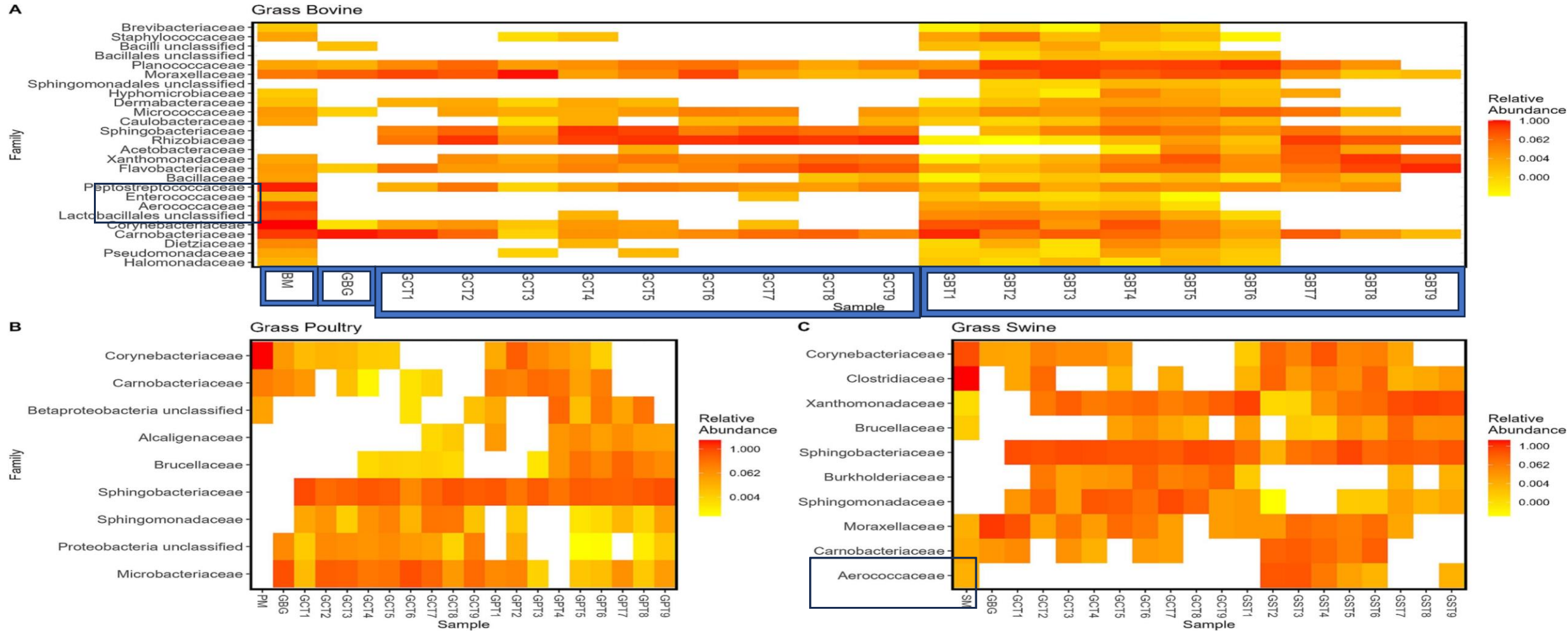
Alpha diversity

- (A) Chao1 index
- (B) Shannon index.
- (C) Non-metric multidimensional scaling (NMDS) ordination plot of 16S rRNA amplicon data.



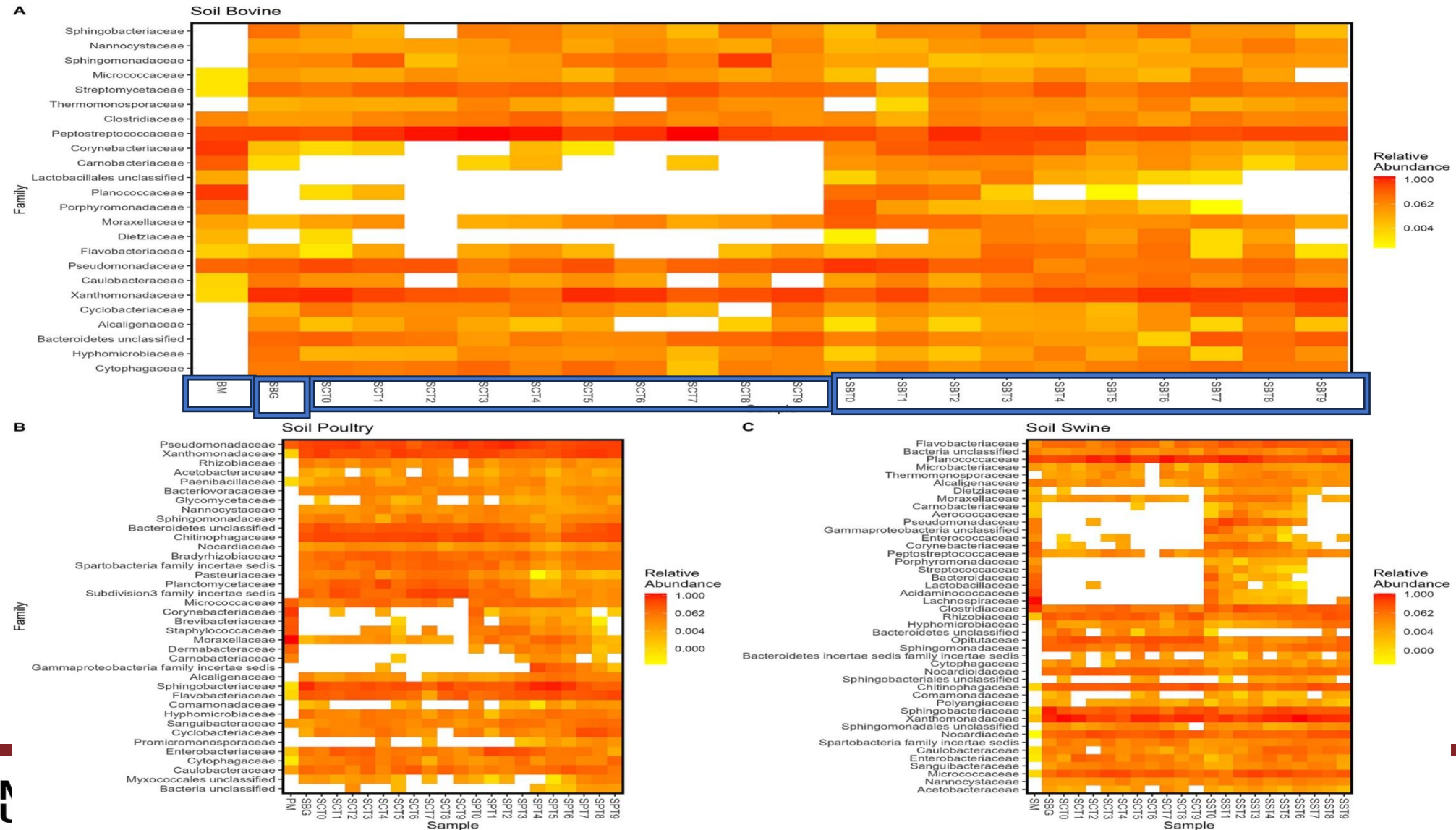
Relative abundances of differentially abundant OTUs

Grass



Relative abundances of differentially abundant OTUs

Soil

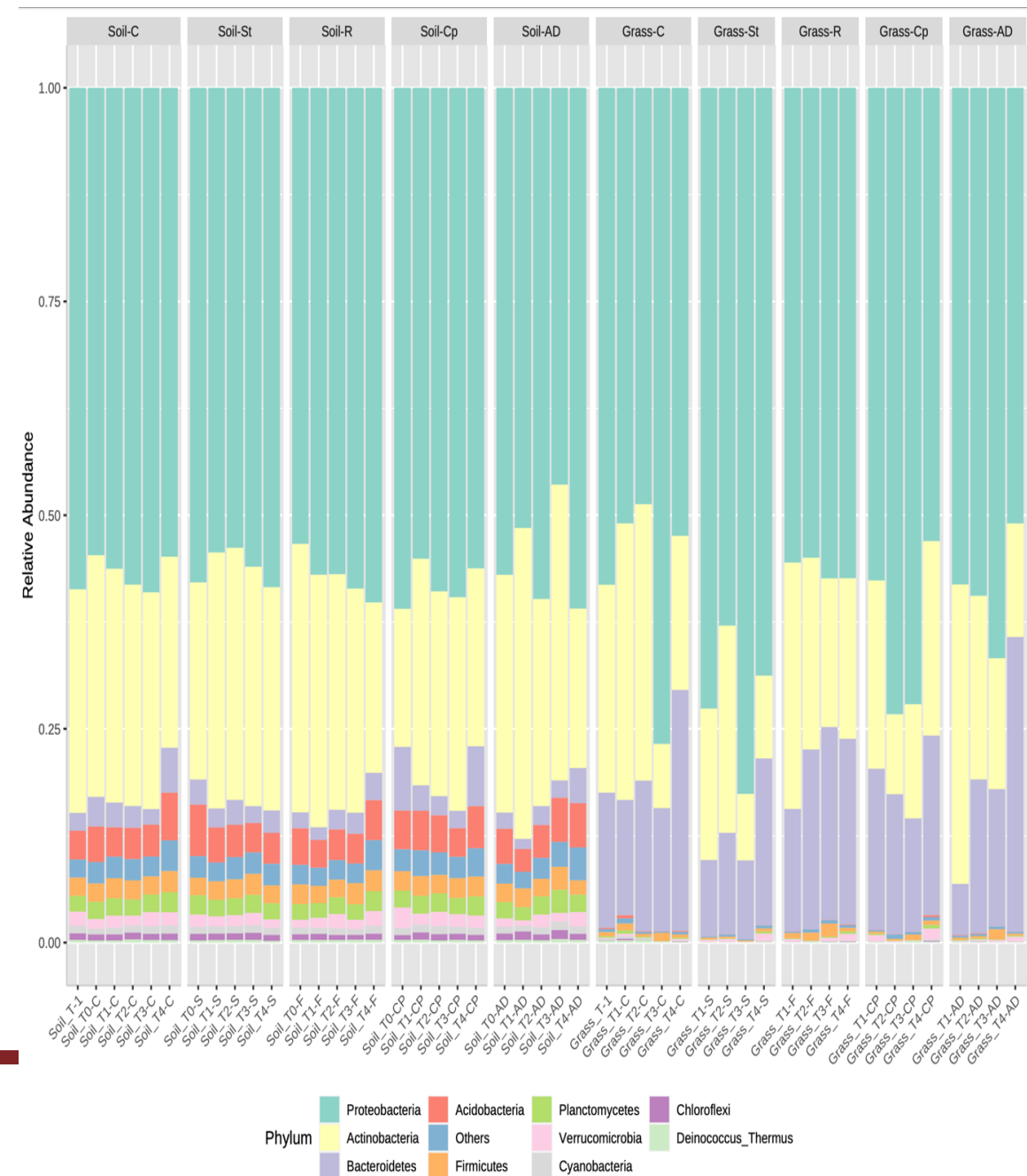
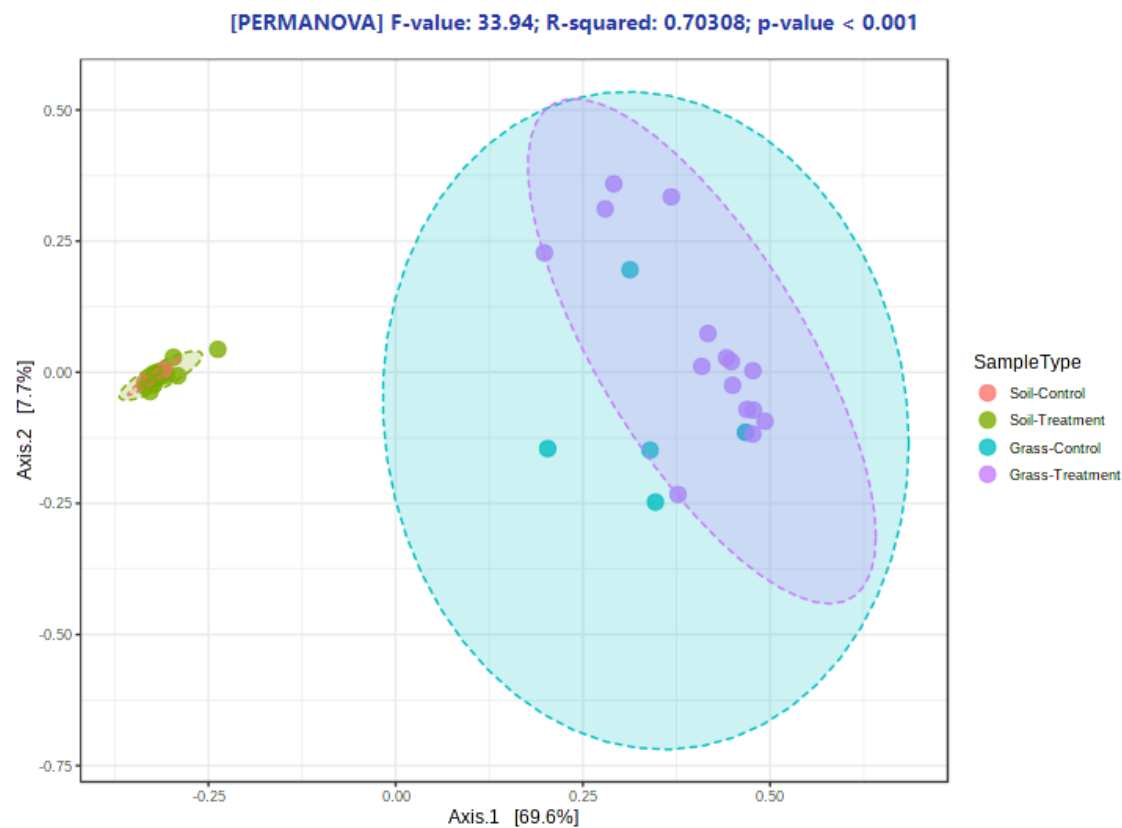


INART study

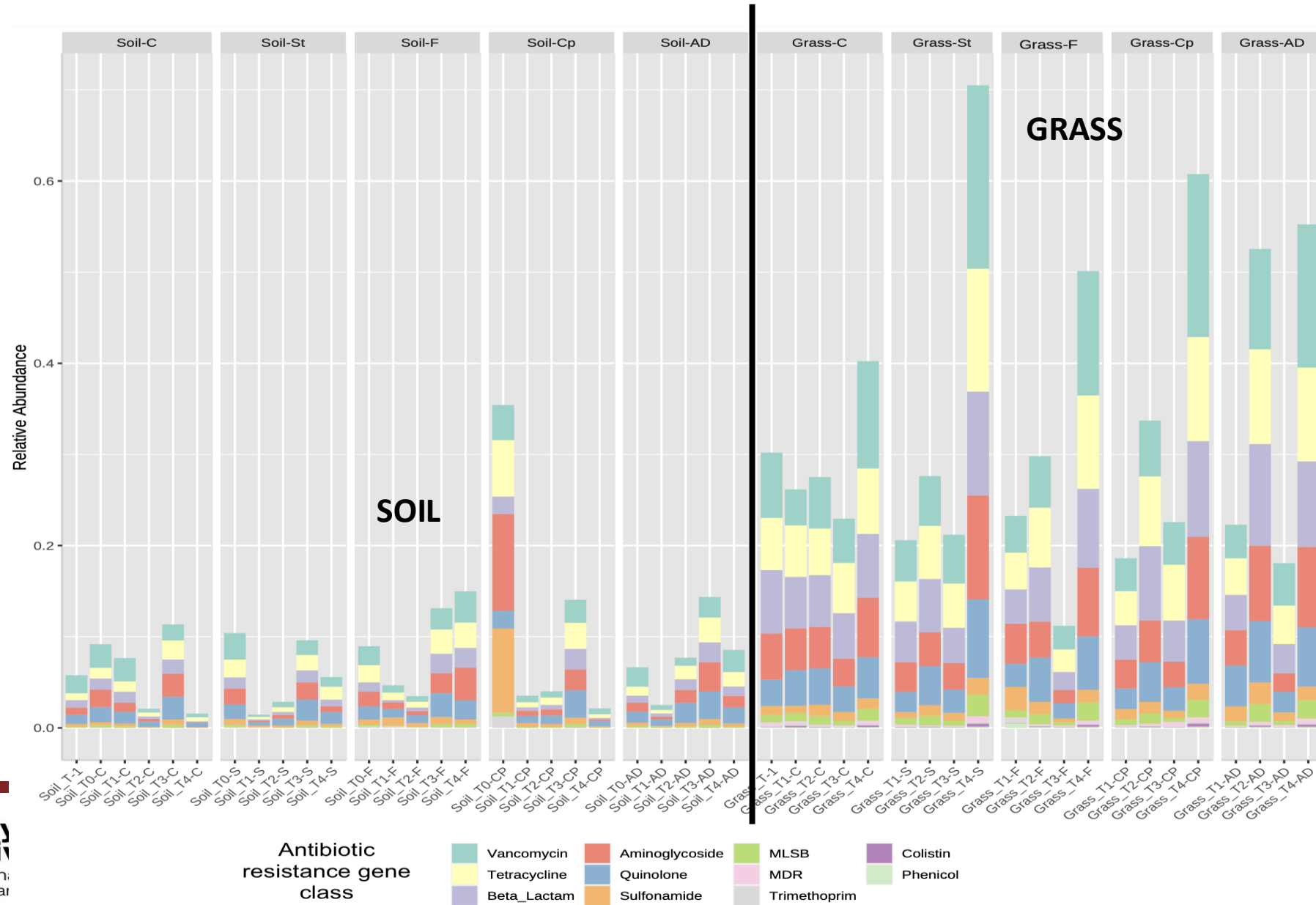
- Treated manure and grassland
- Storage, anaerobic digestion, composted, fresh
- Field trial

INART: Soil & grass field trials

Microbiomes



AMR genes across soil & grass



Soil & Grass DNA analysis

Compared grass and soil microbiomes & resistomes

Soil = known reservoir of AMR genes and bacteria

Grass = unknown

Edible plants – reservoir of AMR genes and bacteria

Grass >>> soil AMR reservoir & direct link to animal via feed

Culture based analysis

- Isolates sampled from grass and soil – field trial 1
- Whole genome sequencing to compare genomes and content
- *Escherichia coli* & *Acinetobacter* spp.

Escherichia coli

Total of 46 isolates

- 7 pig manure (2 ST10 isolates)
39287 & 5
- 14 bovine manure

		Soil	Grass
T0	Directly after manure added		
T1	1 week after manure added	6	4
T2	2 weeks after manure added		
T3	3 weeks after manure added	1	1
T4	4 weeks after manure added		
T5	5 weeks after manure added		9
T6	6 weeks after manure added		
T7	10 weeks after manure added	4	
T8	14 weeks after manure added		
T9	18 weeks after manure added		

Yellow = Culturing Timepoints (n=7)

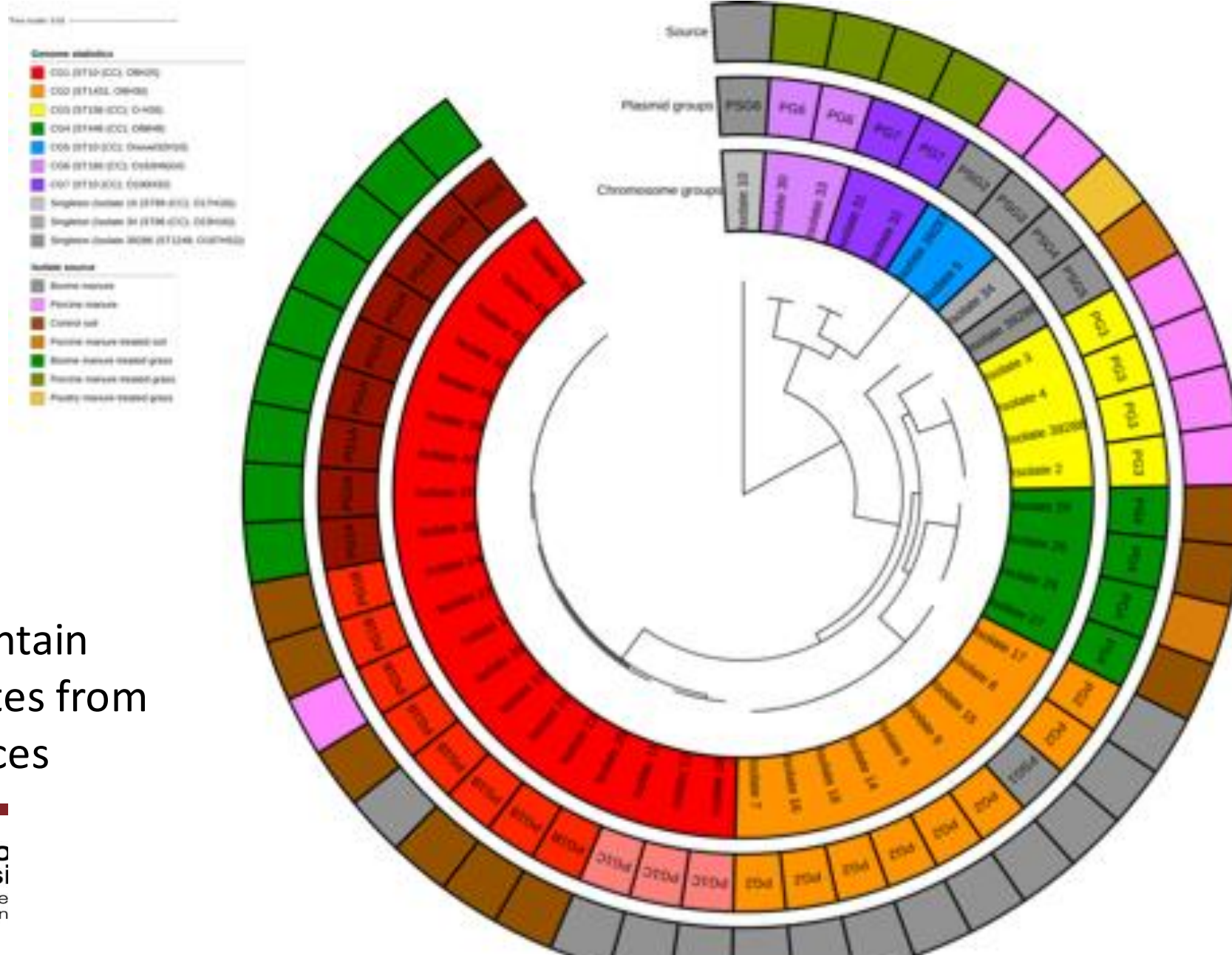
AMR of *E. coli*

Source (n)	Tetracycline	Cefotaxime	Kanamycin	Ciprofloxacin
Manure (21)	16	9	10	15
Soil (11)	6	0	6	0
Grass (14)	13	0	8	1

All susceptible to amikacin and imipenem

No zinc resistance detected although zinc was incorporated into pig feed

Chromosome and plasmid phylogeny



CG1 & CG4 contain identical isolates from different sources

Conclusions

- Identification of the same *E. coli* in pig or bovine manure and grass or soil, indicating transfer and persistence (timeframe)
- Highly related plasmids as well as chromosomes i.e. plasmids were not lost across biomes
- Tetracycline resistance predominated with resistance to three other classes identified
- No carbapenem or amikacin resistance – last line of defence treatments



Acinetobacter baumannii from grass

- *Acinetobacter baumannii* is an opportunistic pathogen of hospital patients
- Natural environment is soil
- AMR of concern – carbapenems & fluoroquinolones

Investigated presence of *A. baumannii* on grass, AMR profiles & similarity to known human pathogens

Same timeframe of sampling as *E. coli*

N = 61 isolates from grass

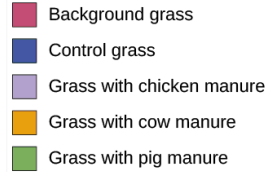
Compared with 148 human isolates, 31 animal isolates

Tree scale: 0.001

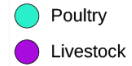
International clones



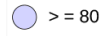
Isolation source (grassland isolates)



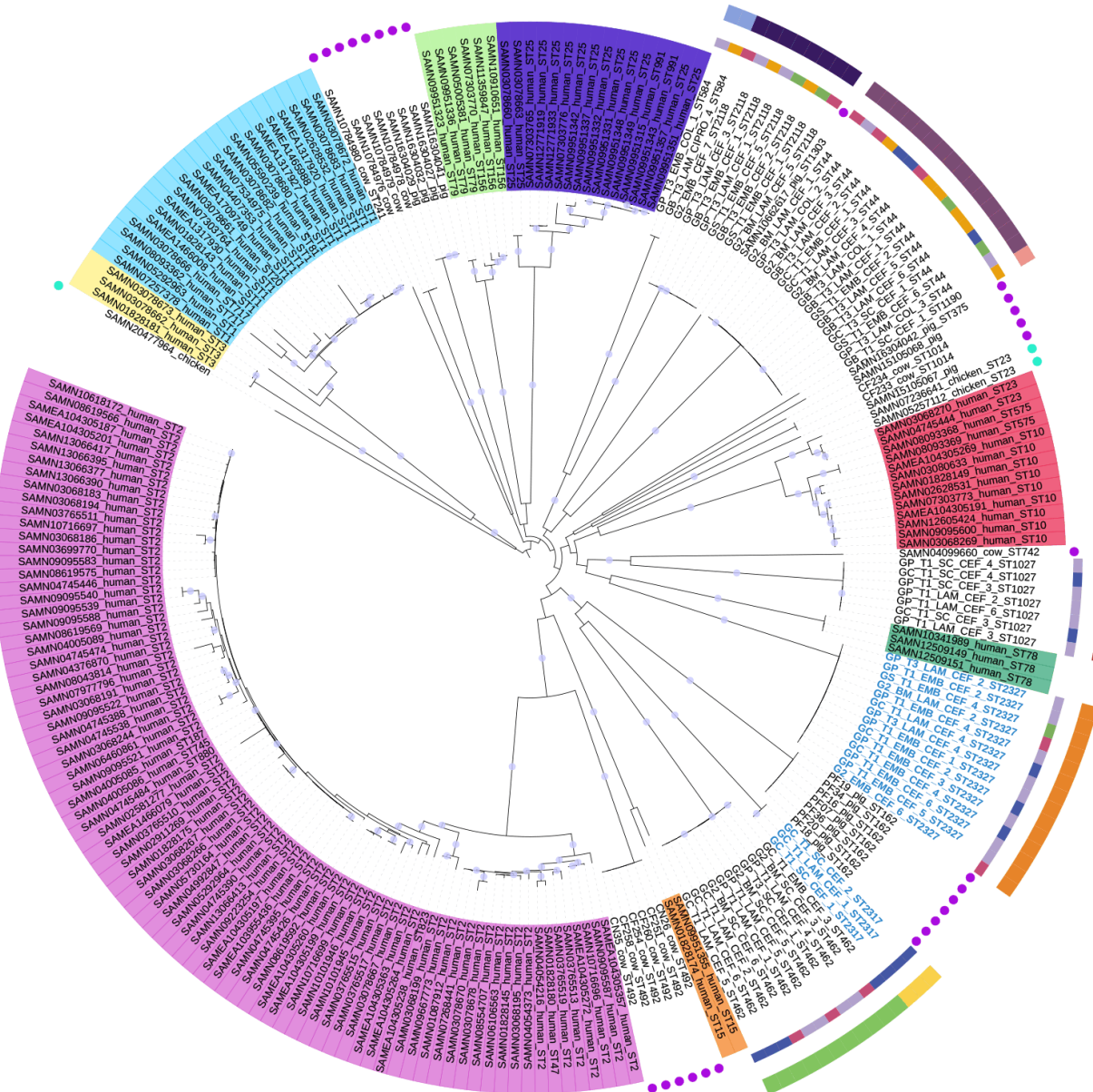
Isolation source



Bootstrap



Grassland isolates groups

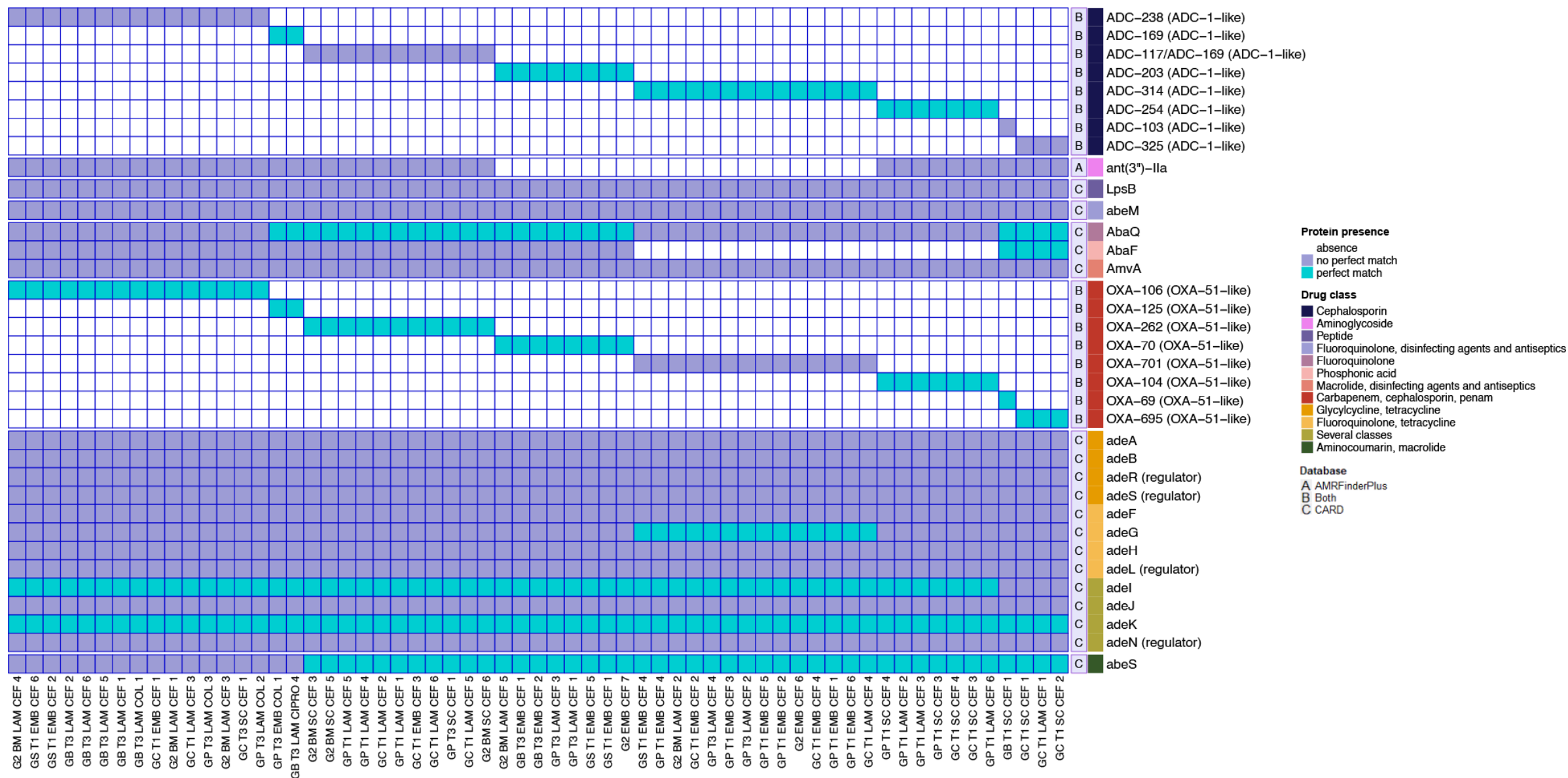


Variety of grass isolation sources but identical *A. baumannii* (inner bar colours)

Group 2: Timepoints across background, T2, T3
Treated with pig or cow manure

Grass is common factor not manure and persist over time

AMR profiles



Conclusions

- Presumption that *A. baumannii* from environment are closely related to hospital isolates – incorrect
- Grass *A. baumannii* are evolutionarily distinct to each other and from human and animal but closer to animal
- Diversity of *A. baumannii* within one field from the grass was as diverse as all *A. baumannii* clones globally and the animal isolates.
- Manure did not play a role in presence i.e. *A. baumannii* are part of the grass microbiome
- *bla*_{OXA-51-like} is the only AMR gene of concern



Overall conclusions

- Grass microbiome and resistome is distinct from soil
- Manure influences soil and grass microbiome for a limited time
- Grass resistome is something to include in environmental testing separate from soil (probably all plants)
- *E. coli* and AMR *E. coli* transfer and persist from manure
- *A. baumannii* on grass are from grass and are evolutionarily distinct from each other, animal and human isolates

Acknowledgements



Dr Fiona Brenna & Dr Kaye Burgess, Teagasc
Staff at Johnston Castle, Teagasc
& Collaborators



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